

AMENDMENTS TO THE CLAIMS

The following claims will replace all prior versions and listings of claims in this application.

1 – 16. (Cancelled)

17. (Currently amended) A method for modifying glycosylation structures on ~~proteins~~ glycoproteins expressed in a eukaryotic host cell comprising: expressing in said host cell a recombinant nucleic acid encoding a polypeptide having an endomannosidase activity that is targeted to a vesicular compartment within the host cell, wherein said nucleic acid encoding a polypeptide having an endomannosidase activity is selected from the group consisting of:

(a) a nucleic acid that hybridizes under stringent conditions to SEQ ID NO:1 or SEQ ID NO:3; and

(b) a nucleic acid that encodes a polypeptide that is at least 75% identical to SEQ ID NO:2 or SEQ ID NO:4.

18. (Currently amended) The method of claim 17 wherein the endomannosidase activity further comprises the activity of truncating $\text{Glc}_{1-3}\text{Man}_{9-5}\text{GlcNAc}_2$ to $\text{Man}_8\text{-}_4\text{GlcNAc}_2$, wherein $\text{Glc}\alpha 1,3\text{Man}$, $\text{Glc}_2\alpha 1,3\text{Man}$ or $\text{Glc}_3\alpha 1,3\text{Man}$ are removed.

19. (Previously presented) The method of claim 17 wherein the endomannosidase activity removes from a glucosylated glycan on proteins expressed in said host cell at least one glucose residue and at least one mannose residue.

20. (Previously presented) The method of claim 17 wherein the endomannosidase is targeted to the endoplasmic reticulum, the early, medial, late Golgi or trans Golgi network within the host cell.

21. (Original) The method of claim 17 wherein the endomannosidase is of host origin but has been modified by mutation, promoter strength or copy number to enhance activity.

22. (Previously presented) The method of claim 35 wherein the endomannosidase is secreted.

23. (Previously presented) The method of claim 17 wherein the host is a mammalian, plant, insect, fungal, yeast, algal or bacterial cell.

24. (Previously presented) The method of claim 17 wherein the host cell is from a eukaryote selected from the group consisting of *Pichia sp.*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium sp.*, and *Neurospora crassa*.

25. (Previously presented) The method of claim 17 wherein expression of the endomannosidase activity modifies a glucosylated glycoprotein that has bypassed the endoplasmic reticulum.

26. (Currently amended) The method of claim 17, wherein ~~the step of expressing said endomannosidase activity comprises transforming a eukaryotic host cell with a nucleic acid molecule capable of expressing a polypeptide exhibiting endomannosidase activity, said nucleic acid molecule comprising or consisting of a nucleic acid sequence~~ is selected from the group consisting of: (a) SEQ ID NO: 1 or 3; and (b) ~~a nucleic acid sequence that is a degenerate variant of SEQ ID NO: 1 or 3;~~ (c) ~~a nucleic acid sequence at least 78% identical to SEQ ID NO: 1 or 3;~~ (d) a nucleic acid sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2 or 4; (e) ~~a nucleic acid sequence that encodes a polypeptide at least 77% identical to SEQ ID NO:2 or 4;~~ (f) a nucleic acid sequence that hybridizes under stringent conditions to SEQ ID NO:1 or 3; and (g) ~~a nucleic acid sequence comprising a fragment of any one of (a)–(f) that is at least 60 contiguous nucleotides in length and retains endomannosidase activity.~~

27. (Previously presented) The method of claim 26 wherein the endomannosidase activity has optimal activity at a pH between about 5.2 and about 7.2.

28. (Previously presented) The method of claim 26 wherein the endomannosidase activity has optimal activity at a pH of about pH 6.2.

29. (Currently amended) The method of ~~claims~~ claim 26 wherein the encoded polypeptide hydrolyzes at least one glucose residue and at least one mannose residue on a Glc₁₋₃Man₅GlcNAc₂, Glc₁₋₃Man₆GlcNAc₂, Glc₁₋₃Man₇GlcNAc₂, Glc₁₋₃Man₈GlcNAc₂, Glc₁₋₃Man₉GlcNAc₂ or glucosylated higher mannan glycan.

30. (Previously presented) A eukaryotic host cell that produces modified glycosylation structures on proteins according to the method of claim 17.

31. (Previously presented) The method of claim 24 wherein the *Pichia sp.* is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, and *Pichia methanolica*.

32. (Previously presented) The method of claim 24 wherein the *Fusarium sp.* is selected from the group consisting of *Fusarium gramineum* and *Fusarium venenatum*.

33. (Previously presented) The method of claim 24 wherein the *Saccharomyces sp.* is *Saccharomyces cerevisiae*.

34. (Previously presented) The method of claim 24 wherein the *Kluyveromyces sp.* is *Kluyveromyces lactis*.

35. (Currently amended) A method for modifying glycosylation structures on ~~proteins~~ glycoproteins expressed in a lower eukaryotic host cell comprising expressing in the host cell a recombinant nucleic acid encoding a polypeptide having an endomannosidase activity, wherein said nucleic acid encoding a polypeptide having an endomannosidase activity is selected from the group consisting of:

(a) a nucleic acid that hybridizes under stringent conditions to SEQ ID NO:1 or SEQ ID NO:3; and

(b) a nucleic acid that encodes a polypeptide that is at least 75% identical to SEQ ID NO:2 or SEQ ID NO:4.

36. (Previously presented) The method of claim 35 wherein the endomannosidase activity further comprises the activity of truncating $\text{Glc}_{1-3}\text{Man}_{9-5}\text{GlcNAc}_2$ to $\text{Man}_8\text{GlcNAc}_2$, wherein $\text{Glc}\alpha 1,3\text{Man}$, $\text{Glc}_2\alpha 1,3\text{Man}$ or $\text{Glc}_3\alpha 1,3\text{Man}$ are removed.

37. (Previously presented) The method of claim 35 wherein the endomannosidase activity removes from a glucosylated glycan on proteins expressed in said host cell at least one glucose residue and at least one mannose residue.

38. (Previously presented) The method of claim 35 wherein the endomannosidase is targeted to the endoplasmic reticulum, the early, medial, late Golgi, trans Golgi network or any vesicular compartment within the host cell.

39. (Previously presented) The method of claim 35 wherein the endomannosidase is of host origin but has been modified by mutation, promoter strength or copy number to enhance activity.

40. (Previously presented) The method of claim 35 wherein the host is a fungal, yeast or algal cell.

41. (Previously presented) The method of claim 35 wherein the lower eukaryotic host cell is from a eukaryote selected from the group consisting of *Pichia sp.*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium sp.*, and *Neurospora crassa*.

42. (Previously presented) The method of claim 41 wherein the *Pichia sp.* is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia*

koclamae, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, and *Pichia methanolica*.

43. (Previously presented) The method of claim 41 wherein the *Fusarium sp.* is selected from the group consisting of *Fusarium gramineum* and *Fusarium venenatum*.

44. (Previously presented) The method of claim 41 wherein the *Saccharomyces sp.* is *Saccharomyces cerevisiae*.

45. (Previously presented) The method of claim 41 wherein the *Kluyveromyces sp.* is *Kluyveromyces lactis*.

46. (Previously presented) The method of claim 41 wherein expression of the endomannosidase activity modifies a glucosylated glycoprotein that has bypassed the endoplasmic reticulum.

47. (Currently amended) The method of claim 35 wherein ~~the step of expressing said endomannosidase activity comprises transforming the lower eukaryotic host cell with a nucleic acid molecule capable of expressing a polypeptide exhibiting endomannosidase activity; said nucleic acid molecule comprising or consisting of a nucleic acid sequence~~ is selected from the group consisting of: (a) SEQ ID NO: 1 or 3; and (b) a nucleic acid sequence that is a degenerate variant of SEQ ID NO: 1 or 3; (c) a nucleic acid sequence at least 78% identical to SEQ ID NO: 1 or 3; (d) a nucleic acid sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:2 or 4; (e) a nucleic acid sequence that encodes a polypeptide at least 77% identical to SEQ ID NO:2 or 4; (f) a nucleic acid sequence that hybridizes under stringent conditions to SEQ ID NO:1 or 3; and (g) a nucleic acid sequence comprising a fragment of any one of (a) (f) that is at least 60 contiguous nucleotides in length and retains endomannosidase activity.

48. (Previously presented) The method of claim 47 wherein the endomannosidase activity has optimal activity at a pH between about 5.2 and about 7.2.

49. (Previously presented) The method of claim 47, wherein the endomannosidase activity has optimal activity at a pH of about pH 6.2.

50. (Previously presented) The method of claims 47, wherein the encoded polypeptide hydrolyzes at least one glucose residue and at least one mannose residue on a $\text{Glc}_{1-3}\text{Man}_5\text{GlcNAc}_2$, $\text{Glc}_{1-3}\text{Man}_6\text{GlcNAc}_2$, $\text{Glc}_{1-3}\text{Man}_7\text{GlcNAc}_2$, $\text{Glc}_{1-3}\text{Man}_8\text{GlcNAc}_2$, $\text{Glc}_{1-3}\text{Man}_9\text{GlcNAc}_2$ or glucosylated higher mannan glycan.

51. (Previously presented) A lower eukaryotic host cell that produces modified glycosylation structures on proteins according to the method of claim 35.

52. (New) The method of claim 17 wherein said nucleic acid encoding a polypeptide having endomannosidase activity is ligated in-frame to a cellular targeting signal peptide.

53. (New) The method of claim 35 wherein said nucleic acid encoding a polypeptide having endomannosidase activity is ligated in-frame to a cellular targeting signal peptide.